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THE TRANSMISSION OF THE ROUS FILTERABLE AGENT TO THE NORMAL TISSUES OF FOWLS.

Edward Mellanby.

Field Laboratory, University of Sheffield.

This publication is an account of experimental work, some of the results of which have been given in summary form in the Annual Reports of the British Empire Cancer Campaign (Mellanby, 1935, 1936, 1937).

In a previous paper (Mellanby, 1938) it was shown that the filterable agent of a Rous sarcoma can pass into a tumour propagated from one initiated by dibenzanthracene or tar, so that two types of tumour can now be produced from it—a dibenzanthracene or tar tumour by inoculation of cells and a Rous tumour by inoculation of cell-free filtrate made from it.

On one occasion an attempt was made to compare the effect of an extract of a dibenzanthracene tumour with that of an extract of heart muscle (free from tumour tissue), taken from a bird which was carrying both dibenzanthracene and Rous sarcomata, and it was noted with surprise that the injection of extract of heart muscle produced a Rous sarcoma. It seemed necessary, therefore, to make similar tests on the other organs of fowls bearing Rous sarcomata, as it had been thought up to this point that the dibenzanthracene tumour, in storing the Rous agent, stood in a category by itself, because it was a tumour with growth properties in many ways comparable to those of the Rous sarcoma. The object of this publication is to show that this is not the case, but that all the organs tested, some more than others, are able to act as depositories of the filterable agent transferred from a Rous sarcoma. It may be emphasised that the organs which were found to contain large quantities of the Rous filterable agent were themselves normal in the sense that they did not contain any observable Rous sarcomatous growth.

That the filterable agent can leave the original Rous tumour and enter the blood stream has already been established. Rous, Murphy and Tytler (1912) showed that if large quantities of centrifuged paraffin plasma collected from fowls moribund with metastatic growths were injected into other fowls, a new sarcoma was produced in some instances. At a later date

Lewis and Andervont (1926) extended these observations by showing that both the plasma and the leucocytes of sarcomatous chickens could act as tumour-producing agents. More successes were obtained by the injection of the leucocytes of such fowls than of their plasma. By the use of blood injections, they carried on the serial transmission of tumours for four generations. They also found that the tumour-bearing fowls need not be moribund, and that the blood carried the infective agent even when there were no metastatic growths. It is of interest to note that these facts not only applied to a Rous sarcoma but also to a tumour grown by Carrel and said to have been originally chemically induced by indol.

Pentimalli (1925 *a* and *b*) has also investigated the subject. He found that the effectiveness of the blood of Rous-bearing fowls to produce new tumours depended on the type of metastases present. If the metastases were red and hæmorrhagic, injection of the blood always produced new tumours ; if white, tumours only resulted in 60 per cent. of cases. He also found that in both tumour-bearing fowls and in fowls which had received an intravenous injection of Rous agent, the active agent fixed itself selectively on to damaged tissue and not on to the normal tissues. Thus, according to Pentimalli, injection into fowls of the previously injured parts of liver and muscle of other fowls which had also received Rous agent intravenously always produced a new tumour, whereas the undamaged parts of the same tissues were ineffective.

It does not seem to have been realised previous to the present work that this entrance of Rous agent into ordinary non-cancerous tissues is a normal occurrence, and that, without the formation of metastases and even in the earlier stages of growth, evidence of the presence of this substance in various organs can be readily obtained. As a matter of fact, in the later stages of a Rous sarcoma, when metastatic formation is present, it is often more difficult to obtain growth by the injection of filtered extracts of normal organs.

Experimental methods.

Fowls bearing Rous tumours in each breast muscle were killed at different intervals after the initial inoculation. Cell-free filtrates were made of various tissues and organs by the following method. In no case was a filtrate made of an organ where there was any macroscopic evidence of tumour metastasis or infiltration.

Approximately 2 g. of tissue were well ground with silver sand in a small mortar and 20 c.c. of saline (0·85 per cent. NaCl in tap water) gradually added and mixed : the extract was centrifuged for 15 minutes at 2000 *r.p.m.* and the supernatant fluid filtered through a small paper-pulp filter, the filter having a depth of 1 in. of paper pulp. Usually the filtration required the assistance of a water pump, two or three inches of mercury pressure being sufficient. When removing the organs, fresh sterilised instruments were used at every stage, and all filters, centrifuge tubes and other vessels were sterilised.

Great care was taken in opening up the fowl to avoid contamination of the different organs by the tumour and to prevent the scissors used for cutting the skin from touching any of the organs to be tested. The four organs chosen for examination in the first experiments were brain, liver, spleen and leg muscle.

Fowls were inoculated in each breast with 1 c.c. doses of paper-pulp

filtrates of Rous sarcoma. After varying periods of time following the original inoculation they were killed and cell-free filtrates made from the organs mentioned. In some cases the *cells* of organs as well as paper-pulp filtrates were used for inoculation.

Experimental results.

In the first place the results of a typical series of experiments will be given.

Experiment 1. A cell-free filtrate from a Rous tumour was injected into the breast muscle of five fowls. These were killed at different times after the initial injection, namely 6, 9, 14, 19 and 22 days respectively. Cell-free filtrates and in some cases the cells themselves of the different organs, leg muscle, brain, spleen and liver, were injected into other fowls. The results are shown in chart 1.

It will be seen that :—

1. No tumour was produced by inoculation of either the cells or the cell-free filtrates of any organ of the fowl killed only 6 days after the original injection of Rous filtrate, but that after nine days' growth many positive results were produced, especially with the cell inoculations.

2. Although the fowl killed 22 days after Rous inoculation had a large metastasis in the lungs, only two tumours were produced out of the 20 inoculations made, both of these being in a fowl injected with splenic filtrate.

3. The cell inoculation of the liver and spleen produced a greater relative number of new growths than the cell-free filtrates made from these organs. The growths produced by the cell injections developed more quickly than those produced by cell-free filtrates. The former were generally palpable about the 10th to the 14th, the latter on the 20th to the 25th day after injection.

Experiment 2. This experiment was made to test the fact, indicated by expt. 1, that the tissue and organ cells of the fowl do not contain demonstrable amounts of filterable Rous agent in the early days after the initial inoculation of Rous cells, at least by the methods adopted. In this experiment only cell-free filtrates of the organs were injected (chart 2).

It will be seen that no tumour was produced in this experiment in the 2- and 6-day inoculation periods, but that a number of tumours appeared in the 10-day-inoculated fowl and that, in the case of the 14-day-inoculated fowl, tumours developed at every site of inoculation.

Another point of interest suggested in expt. 1 is that the normal tissues become less infective again as the period during which the fowl carries a Rous sarcoma lengthens. In general a Rous tumour grows so rapidly that the fowl dies after three weeks. With more slowly growing tumours, however, it may live for five weeks or more.

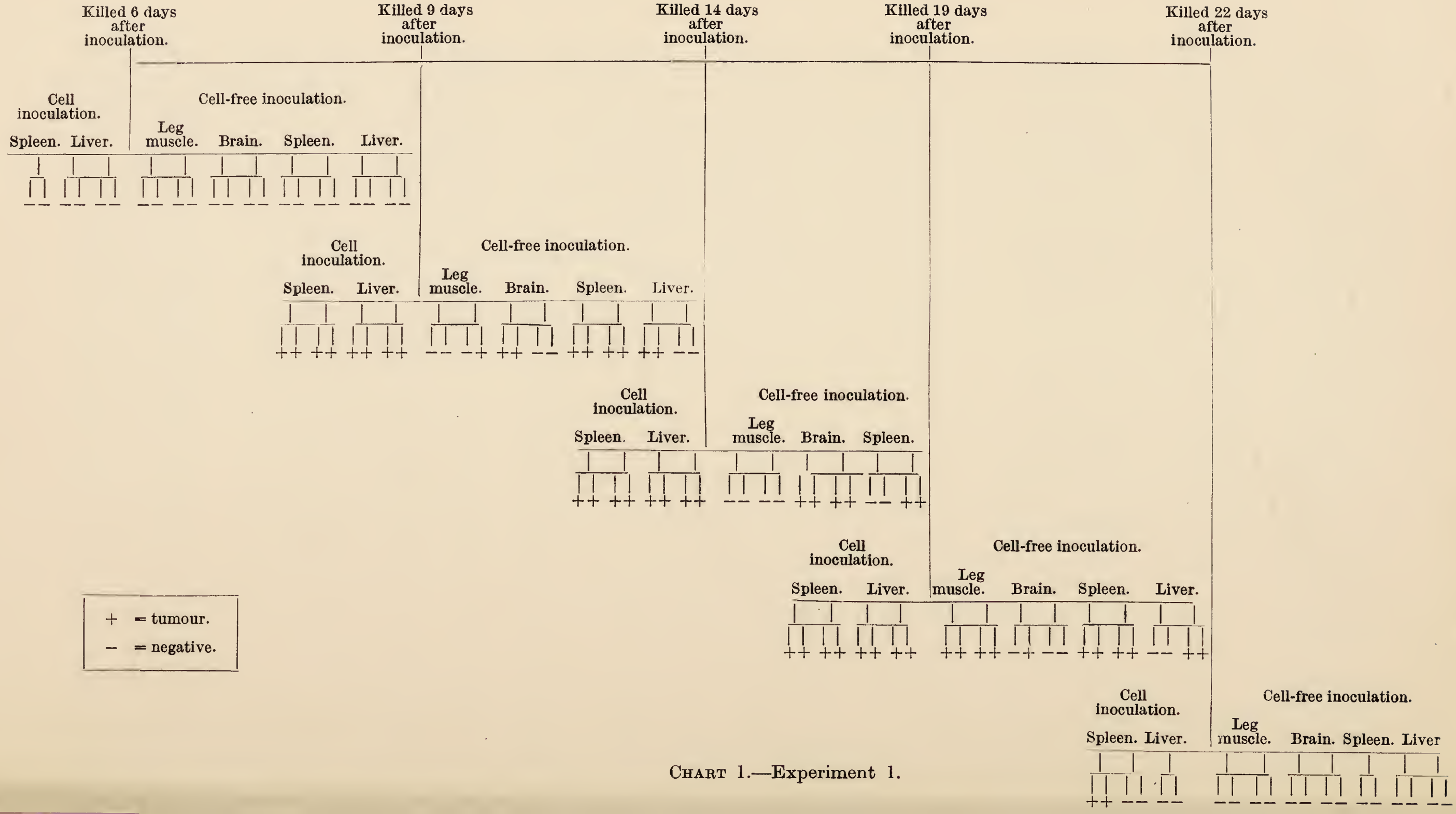

CHART 1.—Experiment 1.

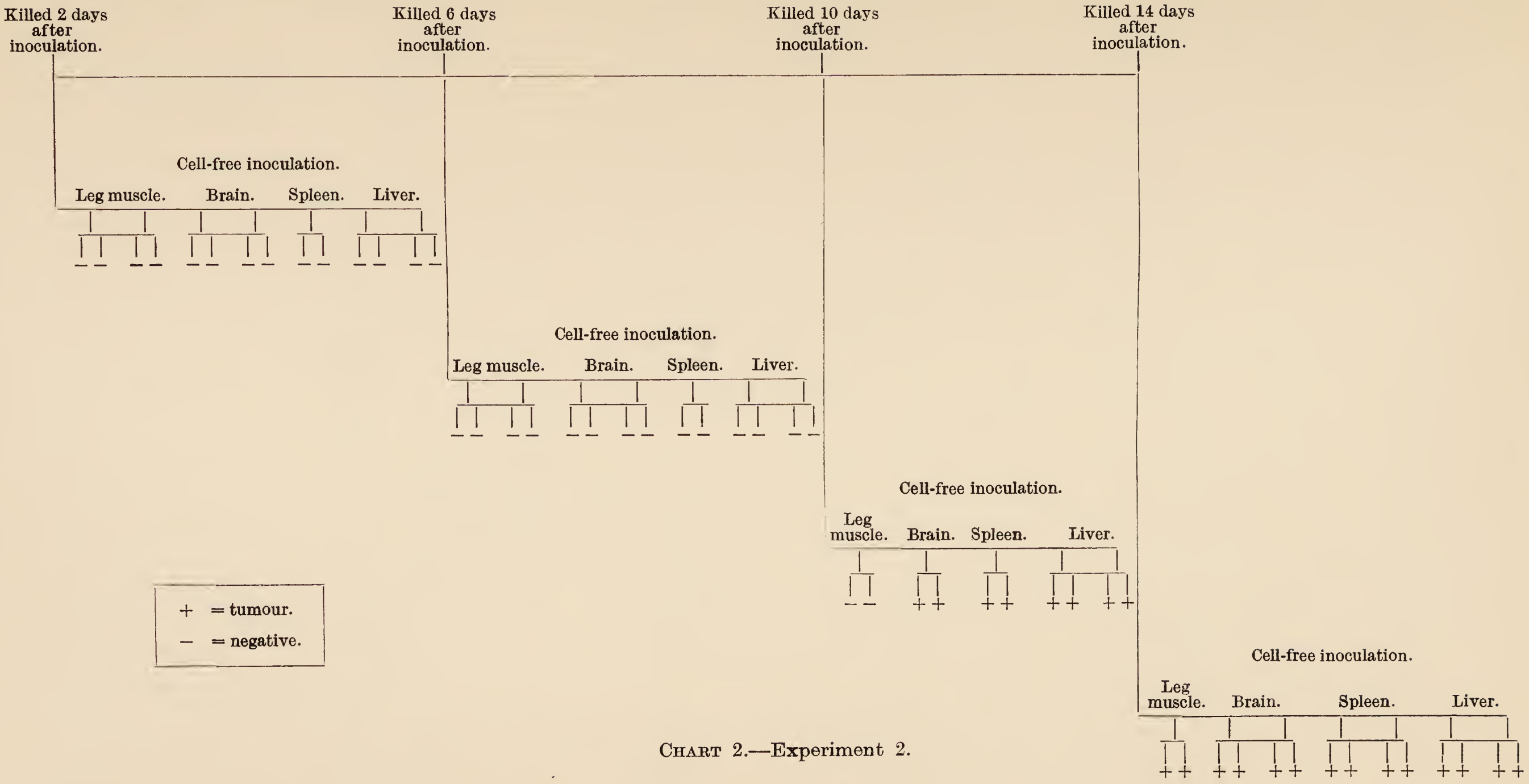

CHART 2.—Experiment 2.

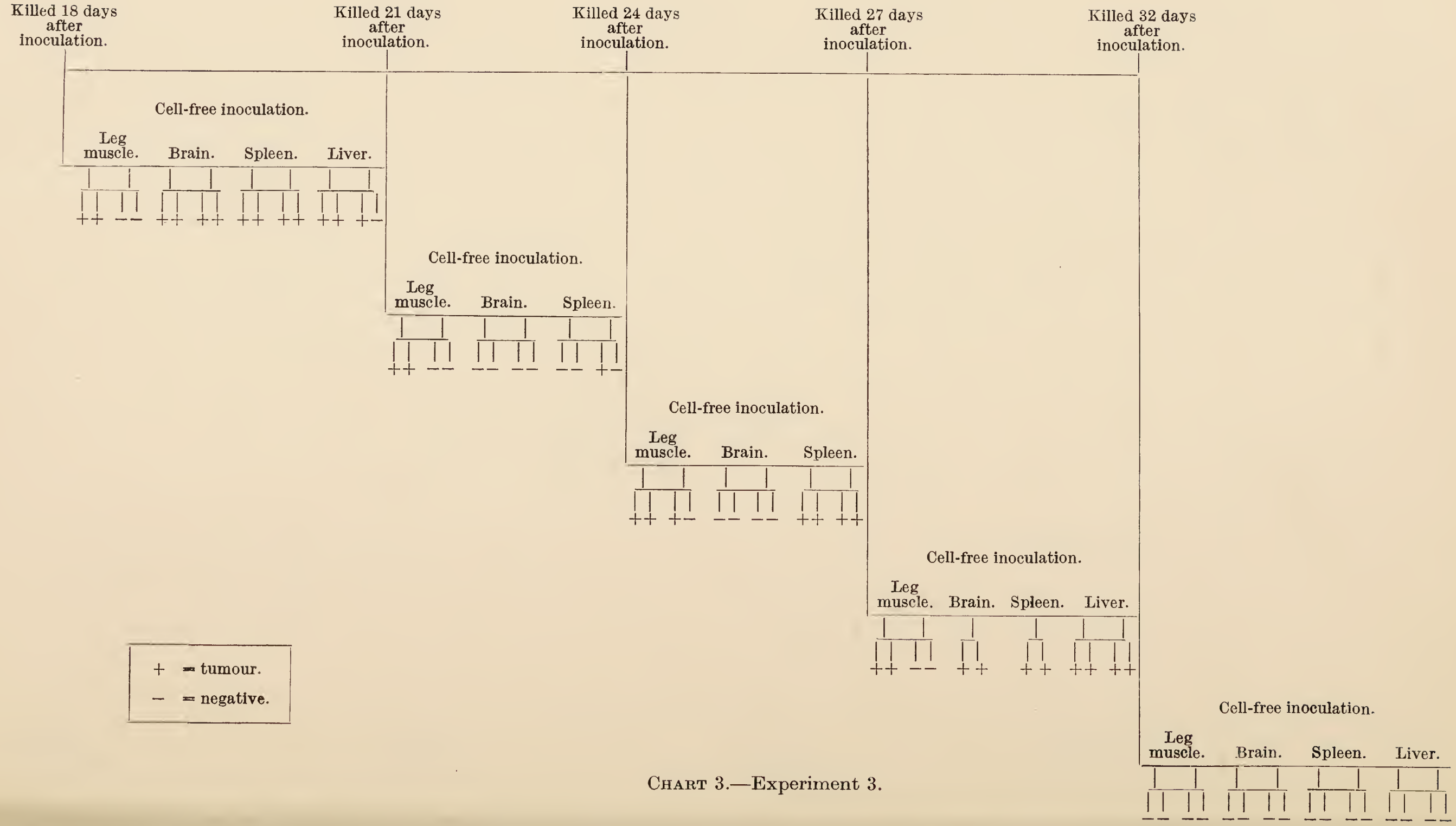

CHART 3.—Experiment 3.

Experiment 3. Out of a large number of fowls inoculated, a selection was made of some which survived longer than usual, *i.e.* those with slow-growing tumours, in order to see whether or no the longer period of survival brought with it a lowered infectivity of the body tissues.

It will be seen (chart 3) that the tissues taken from the fowls inoculated 21, 24 and 32 days previously were less infected with the Rous agent than those taken from the 18-day one. In the case of the 32-day-inoculated fowl, although it had lung metastases, no tumour was produced in the 16 inoculations of cell-free filtrates of its various organs. The 27-day fowl in this experiment is an exception to the general rule as its organ extracts were particularly infective. It may be added that in another similar experiment there were no growths produced in the 16 inoculations of cell-free filtrates of various organs of a 27-day-inoculated fowl. That there is truth in the general statement that the infectivity of the tissues in the later stages of the life of fowls bearing Rous sarcomata is usually reduced can be seen in table I. Whereas in fowls inoculated between 10 and 18 days previously cell-free filtrates of spleen gave 30 tumours out of 48 inoculations (62 per cent.) and of liver 34 tumours out of 46 inoculations (74 per cent.), cell-free filtrates of the same tissues of fowls inoculated 22-32 days previously only produced 7 tumours out of 22 inoculations (32 per cent.) in the case of the spleen and 6 out of 22 inoculations (27 per cent.) in the case of the liver.

Table I summarises the results of the experiments made to test the presence of the Rous agent in cell-free filtrates of the leg muscle, brain, spleen and liver of fowls killed at different periods after inoculation with Rous sarcoma.

TABLE I.

Summary of organ filtrate *experiments.*

No. of days after inoculation when fowls killed.	Tissue filtrates inoculated.							
	Leg muscle.		Brain.		Spleen.		Liver.	
	No. of inocula-tions.	No. of positives.	No. of inocula-tions.	No. of positives.	No. of inocula-tions.	No. of positives.	No. of inocula-tions.	No. of positives.
0-6	16	0	16	0	12	0	16	0
7-9	6	1	8	2	8	6	6	2
10-12	6	2	4	4	10	6	10	10
13-15	10	2	12	8	22	12	20	9
16-18	16	3	10	4	16	12	16	15
19-21	14	8	12	1	14	7	8	6
22-24	16	3	16	4	12	5	10	2
27	8	2	6	2	6	2	8	4
32	4	0	4	0	4	0	4	0
	96	21	88	25	104	50	98	48

Out of a total of 386 inoculations with *cell-free* filtrates 144 or 37·3 per cent. developed tumours. If the first and last groups (*i.e.* the negative groups) are omitted, the successful inoculations were 144 out of 310 (46·4 per cent.). These were made up as follows for the various organs: leg muscle 27·6 per cent., brain 36·7 per cent., spleen 56·8 per cent. and liver 61·5 per cent.

Table II summarises the results of the injection of spleen and liver *cells* of fowls killed at different periods after inoculation with Rous sarcoma.

TABLE II.

Summary of organ cell *experiments.*

No. of days after inoculation when fowls killed.	Tissue cells inoculated.					
	Leg muscle.		Spleen.		Liver.	
	No. of inoculations.	No. of positives.	No. of inoculations.	No. of positives.	No. of inoculations.	No. of positives.
6	...	...	2	0	4	0
9	...	...	4	4	4	4
14	...	...	4	4	4	4
16	...	...	...	...	2	2
17	2	1	2	2	2	2
19	...	...	4	4	4	4
20	2	0	...	...	2	2
22	...	...	4	2	2	0
	4	1	20	16	24	18

Out of the 48 fowl inoculations with *cells*, 35 or 72·9 per cent. developed tumours; or, omitting the 6-day group in which there were no takes, there were 42 inoculations of which 35 were positive (83 per cent.). The percentage of takes in the different organs were: leg muscle cells (only 4 inoculations) 25 per cent., spleen cells 89 per cent., liver cells 90 per cent. Although the liver and spleen were the organs selected for more detailed study in the present work, Rous sarcomata have also been produced by the injection of cells (and cell-free filtrates when tested) of many other organs including brain, heart muscle, kidney, bladder, pancreas and gizzard.

The presence of Rous agent in the dried organs of fowls carrying Rous sarcomata.

It is well known that when a sarcomatous growth of the Rous type is carefully dried and ground to a powder, the powder may retain indefinitely the power to initiate new growths when injected into fowls. In view of the foregoing results, in which it was shown

that tissues of fowls carrying Rous sarcomata could also initiate new-growths when either the cells or the cell-free filtrates were injected, it seemed of interest to determine whether these tissues also retained this property after drying and powdering. The following experiments show that desiccated and ground spleen and liver of fowls carrying Rous sarcomata can produce new-growths on injection.

Experiment 4a. A routine fowl with Rous sarcomata was killed 14 days after inoculation, and the spleen and a part of the liver were removed. These tissues were minced finely with scissors, spread out in thin layers in Petri dishes, and placed in a desiccator over phosphorus pentoxide for ten days. Each dried tissue was then ground up in a mortar. Normal saline made up with tap water was added to the dried powders to give suspensions of approximately such strength that 1 c.c. of each suspension was equivalent respectively to 0·1 g. of fresh spleen tissue, and 0·5 g. of fresh liver tissue. The difference in the amount of dried material injected was due to the difficulty of getting more splenic material.

Four normal fowls were injected in each breast with 1 c.c. of the spleen powder suspension and 4 with 1 c.c. of the liver powder suspension. Of 8 possible tumours in the spleen powder fowls 6 were produced; of 8 possible tumours in the liver powder fowls only 2 were produced, both in the same bird.

Experiment 4b. In a second similar experiment, using the tissues of a fowl with Rous sarcomata 11 days after inoculation, 5 tumours were produced in 4 fowls as the result of 8 inoculations with spleen powder, and 4 tumours in 4 fowls receiving 8 inoculations with liver powder.

These results leave no doubt that the organs of Rous tumour-bearing fowls retain their tumour-producing properties after drying and grinding, just as does the Rous tumour itself.

A number of questions were raised by the foregoing results, including the following. (1) Did the Rous agent, after being produced in and disseminated by the Rous tumour, actually enter the cells of the normal tissues ? (2) If so, was this Rous agent in such tissues destroyed or made ineffective either by the direct metabolic processes of the cells or by the production of antibodies, and if so at what rate ?

It seemed possible to get evidence on the first question by injecting the agent intravenously into fowls and, after an interval, washing out the vascular system by Ringer's solution and testing the washed tissues and the blood itself for the presence of the Rous agent. It is true that positive results obtained from the washed tissues would not be conclusive, for they would not exclude the possibility that the Rous agent might be present in the lymph. On the whole, however, such positive results would favour the view that the agent is present in the cells and not in the blood or other fluids. The following experiments were made to test this point.

The presence of Rous agent in normal tissues of fowls after washing out the blood by perfusion with Ringer's solution.

Experiment 5. In the following experiment 2 fowls were injected intravenously with 10 c.c. of Rous cell-free filtrate, and were perfused with 2 litres of Ringer's solution 24 hours and 3 days respectively after injection. Before perfusion, blood was collected from each fowl. The presence of Rous agent was tested by injecting 0·25 c.c. of whole blood and 0·05 c.c. of spleen cells into other fowls with the results shown in chart 4.

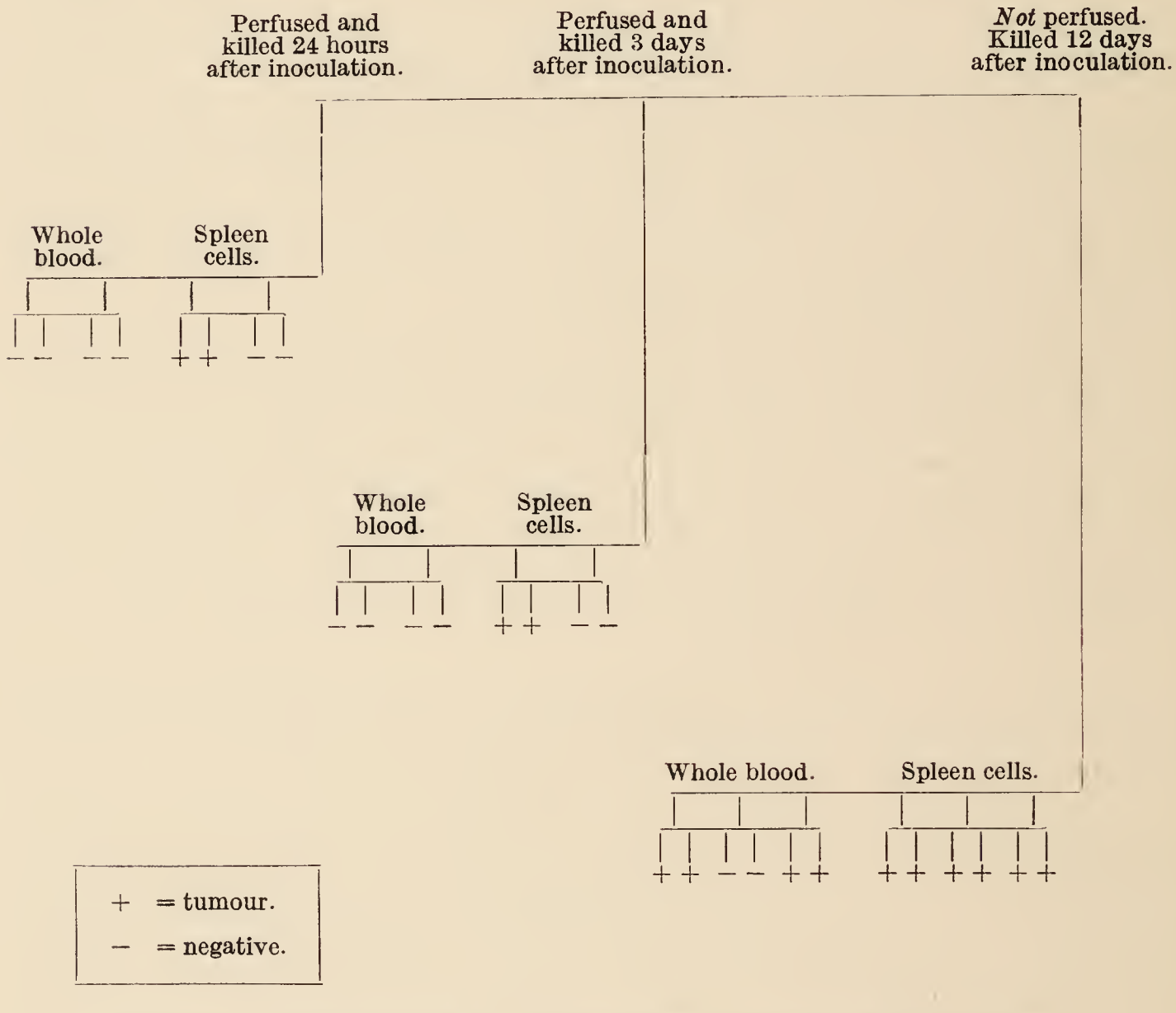

Chart 4.—Experiment 5. Intravenous injection of Rous agent (with and without later perfusion).

These results show that, when Rous agent is present in the fowl, washing out the blood does not deprive the cells of the spleen of their power to induce new-growths. In the same experiments, however, the blood does not give evidence of containing Rous agent when it has been injected intravenously. These results suggest that, after intravenous injection, the Rous agent leaves the blood for the most part and enters the cells of the spleen and liver and probably of other organs.

The last fowl in this series is of interest. It was not perfused, and the tests for the presence of Rous agent were made 12 days after the initial intravenous injection of the Rous cell-free filtrate. By this time a trace of malignant tissue, only detectable by close

examination, had developed at the site of inoculation, so that any Rous agent present may have come from two sources: (1) the original agent injected, and (2) that produced by the minute new-growth which had developed. In this case it will be seen that Rous agent was present not only in the spleen but also in the blood. The Rous agent in the blood might be regarded, in view of its probable absence from the blood in the fowls in which tumours did not develop, as coming directly from the small amount of new-growth at the seat of injection. In view of the minute amount of this new Rous tissue, however, it is difficult to understand how this apparently large amount of Rous active agent in the spleen and blood could have come from these cells. This result, indeed, raises the question as to whether it is not possible for the Rous agent to multiply in the fowl, independently of tumour growth, under some conditions.

Experiment 6a. Two fowls were injected intravenously with 10 c.c. of a paper-pulp filtrate of Rous sarcoma: 24 hours later the fowls were perfused with Ringer's solution, two litres being passed through the circulation of each at a temperature of 37° C. The spleens and livers were then removed, finely minced with scissors and about 0·05 c.c. of the cells injected into each breast of two fowls. Before perfusion, some of the whole blood of these fowls was also taken and 0·25 c.c. injected into the breast of two fowls.

The results were as follows: spleen cells produced 6 tumours out of 6 inoculations in 3 fowls; liver cells produced one tumour out of 6 inoculations; blood produced no tumours.

Experiment 6b. In another experiment where two fowls were injected intravenously with 10 c.c. of cell-free filtrate from a Rous sarcoma and were killed one hour later, and the blood washed out by Ringer's fluid, injection of spleen cells produced two tumours in one bird, the remaining three fowls giving negative results.

These experiments and others of a similar nature support the view that, after the intravenous injection of the Rous agent, this substance is rapidly taken up by the cells of the spleen. Why the liver cells did not, in expt. 6a, give more evidence of the presence of the Rous agent cannot be stated, because earlier quoted experiments show that the liver is a repository of Rous virus equally with the spleen under similar conditions.

The survival time of Rous agent in the tissues of fowls.

Having obtained some evidence, even though not conclusive, that the Rous agent after entry into the blood stream was rapidly taken up by the cells of the spleen, it was now desirable to see if any answer could be given to the second question, namely, whether the Rous agent was destroyed by the direct metabolic activity of the cells into which it had entered, or whether it was neutralised by antibodies in the humoral fluids, and if so at what rate. It

seemed unlikely that the infective agent remained for long periods (*i.e.*, of the order of many weeks) in the cells because, as seen in previous experiments, the later the tests were made in the life of fowls bearing Rous sarcomata, even when metastatic growths were present, the greater was the difficulty in finding the Rous agent in organs like the liver and spleen. In view of the fact that the Rous agent must have been produced and probably released in larger amounts as the tumour grew, it seemed likely that this lowered infectivity of the tissues could only have been the result of some increase in the mechanism of antagonising or destroying the Rous agent.

It was impossible to decide this question by studying the presence of the Rous agent in the spleen or liver of fowls bearing Rous sarcomata, for its presence might signify only that the rate of entry of the Rous agent into these tissues was greater than its rate of disappearance. Some answer might be given to the question if the presence of the Rous agent could be followed in the tissues of birds which had received one intravenous injection of the Rous agent. Even in this case the possibility that the agent after injection increased in amount might prevent any certain deduction from the results. To trace the presence of the Rous agent after a single injection into fowls was clearly the first objective, and this has been done in the following experiments. In the first series fowls received intravenously doses of Rous agent and after varying intervals up to a week were tested for the presence of the agent in the spleen.

The presence of Rous agent in the spleen for varying periods after one intravenous injection.

Experiment 7. Six fowls were each inoculated with 5 c.c. of a paper-pulp filtrate of a Rous sarcoma into the vein of the left wing. They were killed at the following intervals after the injection : 6 hours, 24 hours, 2, 3, 5 and 7 days. After death, cell and cell-free-filtrate inocula were made from the spleen of each fowl and injected into the breasts of other fowls, with the results shown in chart 5.

It will be seen that Rous agent was present in the spleen *cells* of every fowl which had received the intravenous injection of Rous agent. The cell-free filtrates made from the spleen, however, only produced tumours in the case of the 6-hour and 5- and 7-day interval fowls. The results from the 7-day interval fowl had better be disregarded in the present instance, as a tumour was just beginning to grow in the left wing at the site of intravenous injection. In this case, therefore, the presence of the Rous agent in the spleen might have been wholly or partially due to transmission from the new-growth. In all the other fowls there was no evidence of a Rous growth, and any Rous agent in the spleen was

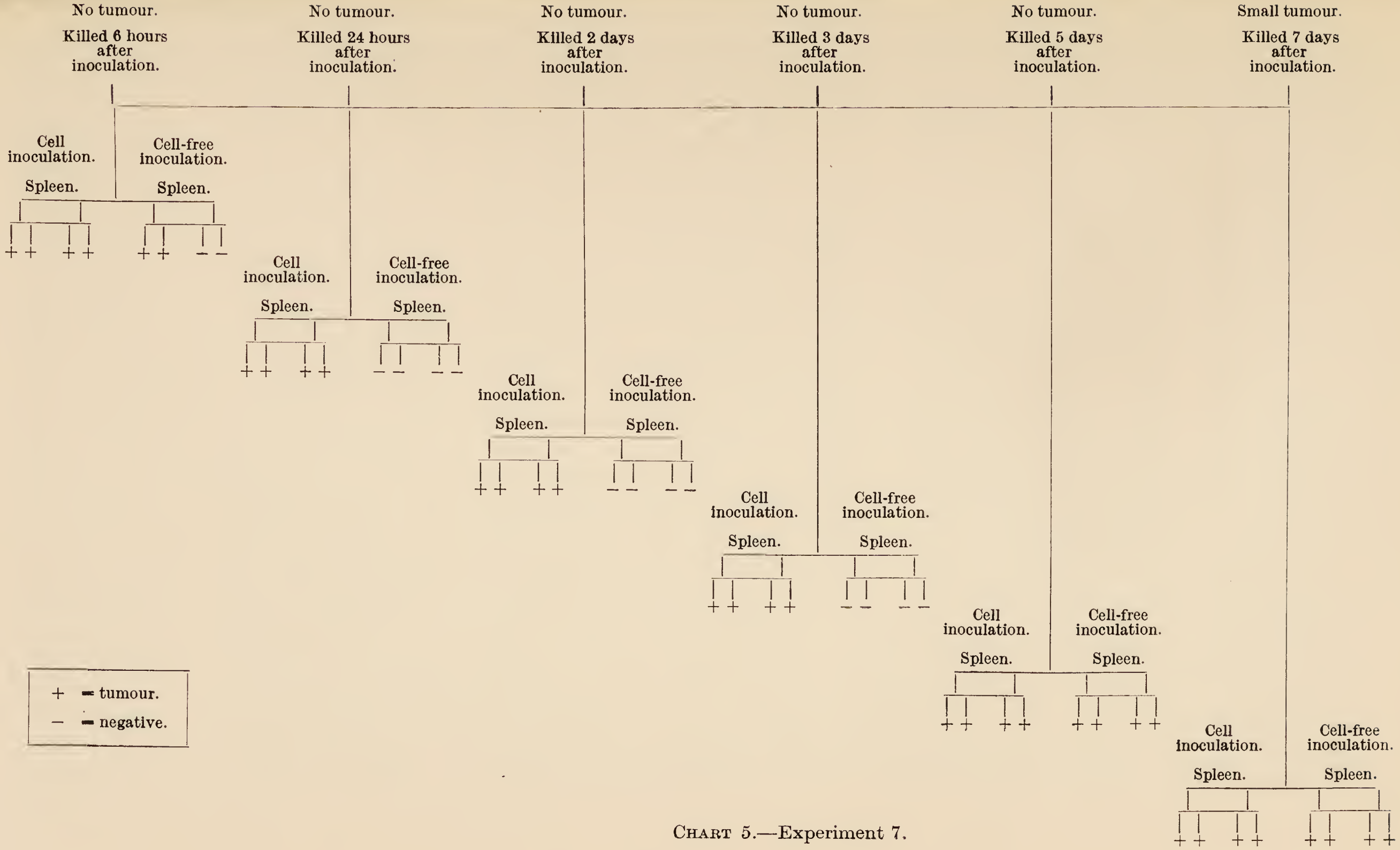

CHART 5.—Experiment 7.

probably the same as that injected, although the possibility that the agent initially injected might have multiplied cannot be excluded. On the whole, however, the deduction can probably be made from these results that when Rous agent is injected intravenously into a fowl, it enters the cells and remains unreduced in amount for at least five days.

In order to gain further information as to the survival time of the Rous agent, a large number of fowls received intravenous injections of this substance and an attempt was made to destroy the tissues in the immediate neighbourhood of the injection with the object of preventing local tumour growth, since the presence of a Rous tumour prevented any deduction being made as to the survival time of the injected Rous agent. In two cases only among this group did fowls not react and produce a tumour, and these fowls were allowed to survive 16 and 41 days respectively after injection.

The absence of Rous agent from the tissues of fowls resistant to intravenous injection of the agent.

Experiment 8. Six fowls were each injected intravenously with 10 c.c. of a paper-pulp filtrate of Rous sarcoma. Five of these fowls died with tumours, but the sixth, which did not develop a tumour, was killed 41 days after the injection. It is evident that the cell-free filtrate injected was rich in Rous agent. Cells of the spleen and liver, also whole blood clot and serum of this fowl, were injected respectively into the breasts of 12 other fowls, but all remained negative. These results indicate that, after an interval of 41 days from the time of injection into a resistant fowl, the Rous agent is destroyed or neutralised.

Another fowl injected intravenously with 10 c.c. of cell-free filtrate which proved to be very active in other fowls of the series failed to respond by the production of a tumour. It was killed after 16 days. Cells of the spleen and liver and also whole blood were injected into other fowls but in no case was a tumour produced.

It might be deduced from these experiments that the Rous agent, when injected intravenously into fowls, was normally destroyed in less than 16 days unless a tumour was produced. The interpretation of these two particular results, however, may be different, and it may be that their resistance to the action of Rous agent was due to the destruction of the agent as soon as it entered the body. It would clearly be a matter of some difficulty to settle a question of this nature.

Discussion.

The observation that an injection of extract of heart muscle of a Rous tumour-bearing fowl gave rise to a Rous sarcoma led to the present work, in which it is shown that the Rous agent is widely diffused throughout the tissues and organs of such fowls and can be easily demonstrated. In practice, therefore, it is not essential to

use Rous cells or their extracts to produce a new growth, since cells of the liver and spleen, and to a less extent of muscle, brain and many other tissues of a Rous tumour-bearing fowl, are also capable of initiating new-growths. As in the case of Rous sarcoma itself the tissues can be dried and powdered and still retain their tumour-producing properties.

The evidence also indicates, although it is not conclusive, that the Rous agent, after being liberated from the Rous tumour or injected intravenously, enters the actual cells of the organs of the body, since washing out the blood with Ringer's solution does not deprive the tissues of the power to induce new-growths.

An attempt was made to find out how long the Rous agent remained in an active form in the spleen of a fowl after a single intravenous injection. Virus was certainly present up to the fifth day, but the development of new tumour tissue with the liberation of more Rous agent prevented further deduction being made after this time. Indirect evidence, however, such as the greater difficulty in producing new-growths by injection of cells or cell-free filtrates of spleen and liver of Rous tumour-bearing fowls after three weeks, even when metastatic growths are present, indicates that a defensive mechanism which antagonises the Rous agent develops and is powerful after a month. Since, however, fowls with Rous tumours usually die about three weeks after the initial injection of Rous tumour cells, the defensive mechanism seldom gets a real chance of being effective.

Two fowls which had resisted a large intravenous injection of Rous agent and remained without tumours did not apparently have any store of agent in the tissues when tested 16 and 41 days respectively after the injection. No deduction can be made from these experiments as to the duration of injected agent in normal fowls, as the unnatural resistance of these two fowls may indicate the presence of a powerful destructive mechanism when the injection was made.

The question of the time in which antibodies to the filterable tumour-exciting agents develop has been studied by Ledingham and Gye (1935). These workers obtained filtrate deposits of Fujinami's myxosarcoma with the aid of a high-speed centrifuge, and made a suspension from these deposits. The development of agglutinins to this suspension was then followed in the serum of fowls bearing Fujinami tumours. The general result was that agglutinins were usually demonstrable during the actual growth of the tumour and appeared about the second week after inoculation. It would be interesting if the Ledingham and Gye technique for demonstrating agglutinins in serum were carried out with the serum of Rous tumour-bearing fowls in association with experiments of the type described in the present publication. If there

were any relation between the development of agglutinins and the amount of Rous agent in the tissues, it might help to throw light on the problem as to whether the mechanism developed in the fowl for inactivating Rous virus depended on the production of agglutinins.

One or two further remarks are worth making in connection with these experiments. The first is that tumours produced by injection of liver, spleen and other cells, when the same technique is applied in all cases, usually take a longer time in starting than tumours produced by injection of Rous sarcoma cells. The tumours produced by cell-free filtrates of such organs are again slower in starting than those produced by injection of the cells of the organs. Another point which might prove of interest on further investigation is that tumours produced by cells of organs or their cell-free filtrates are usually more compact than those initiated by Rous tissue itself. On the other hand, injection of these harder and more compact tumours usually gives rise to the typical loose myxomatous Rous tumours. It may be that these differences of texture and rate of initiation are merely due to the smaller amount of Rous agent injected as compared with that introduced when Rous cells themselves or cell-free filtrates made from them are used.

The results of this work again raise the question of the relation between the Rous agent and the production of tumours. Why is it that tumours do not develop everywhere when the tissues contain such rich stores? There is a general belief that, apart from the presence of the Rous agent, another factor such as local injury is necessary for the initiation of a new-growth. Against this is the fact that, after intravenous injection of Rous agent, new-growths often begin in various parts of the body apart from the tumour at the point of injection. In a series of 10 fowls injected intravenously with Rous agent, growths developed in the lungs of 7 fowls, in the liver of 4 fowls, in the heart of 6 fowls, in the spleen of 4 fowls, and singly in various other places such as the gizzard, thigh and belly wall. In one fowl of the series no tumour grew. In most fowls thus treated a tumour is also produced at the site of intravenous injection.

It is difficult to believe that all these seats of origin of Rous tumours, apart from the point of injection, were injured in any way. On the other hand, many attempts have been made to localise tumour growth to points of injury following intravenous injection of Rous agent and only rarely with success.

It cannot be stated what, if any, is the determining factor for the production of a Rous sarcoma in addition to the presence of the Rous agent. It would appear, however, that a Rous tumour is liable to arise at any point in the body where the agent reaches a certain concentration in proximity to connective tissue cells, and it

may be that this level of concentration varies from organ to organ. Equally certain is it, as is shown by the present work, that many organs of the fowl can contain easily demonstrable amounts of Rous agent without either being stimulated to the production of a Rous tumour or even, apparently, suffering any observable metabolic disorder.

Summary.

1. When a fowl has a Rous sarcoma, the Rous agent is widely diffused throughout the body.

2. The presence of this factor can often be demonstrated in the spleen, liver, muscle, brain and other organs by the production of Rous sarcomata when the cells or cell-free filtrates of these organs are injected into other fowls.

3. It is unusual to find evidence of Rous virus in the organs and tissues of a Rous tumour-bearing fowl until the seventh day after injecting the Rous cells, *i.e.* until the new growth is established.

4. It is easiest to demonstrate the presence of Rous virus in the organs of a Rous-bearing fowl between the 10th and the 18th day after injection.

5. As a rule it is again more difficult to demonstrate the presence of Rous virus in liver, spleen, etc., at a later period than the 20th day, *i.e.* when the fowl may be approaching death, even when metastases are present. A method of antagonising the Rous agent often seems to have developed by this time.

6. The spleen and liver of Rous tumour-bearing fowls can be dried and ground and still retain their tumour-inducing properties on injection ; thus resembling in this respect the Rous sarcoma itself.

7. After washing out the blood of Rous sarcomatous fowls, or of fowls which have received an intravenous injection of Rous agent, with Ringer's solution, the injection of cells of spleen and liver may still induce growths in other birds, indicating that the Rous agent enters the cells of these organs.

8. On giving one injection of a cell-free filtrate of Rous sarcoma intravenously to fowls, the presence of the Rous virus can be demonstrated in the spleen at all periods up to 5 days, suggesting that the virus injected is not reduced in amount or in effectiveness in this time. It is also present in the spleen after 5 days, but the usual initiation of a new growth at the site of injection about this time prevents further deductions being made as to the fate of the initially injected Rous agent. In two resistant fowls no Rous agent could be found in the tissues 16 and 41 days respectively after intravenous injection.

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